Revista Mexicana de Ciencias Agrícolas
DOI: https://doi.org/10.29312/remexca.v16i3.3614
Investigation note

Iluminación LED y crecimiento *in vitro* de palma de jipi

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Abstract

The toquilla palm (*Carludovica palmata*) is a species in high demand for the production of handicrafts of high economic value in southeastern Mexico. There are no methodologies for intensive cultivation of the species and artisanal production depends on key phenotypic characteristics. *In vitro* culture with LED light promotes micropropagation. This study, carried out in Chiná, Campeche, Mexico in 2023, assessed the growth of toquilla palm plants under different treatments of LED light (red, blue, blue/red, and white), white fluorescent light, and darkness. A protocol for *in vitro* germination of seeds was optimized and the effect of kinetin (KIN) in the multiplication phase was evaluated. Plants under blue/red LED lights elongated significantly, whereas those exposed to red LEDs developed a better root system, making these arrangements optimal for large-scale toquilla palm production.

Palabras clave:

Carludovica palmata, in vitro culture, LED light, toquilla palm.

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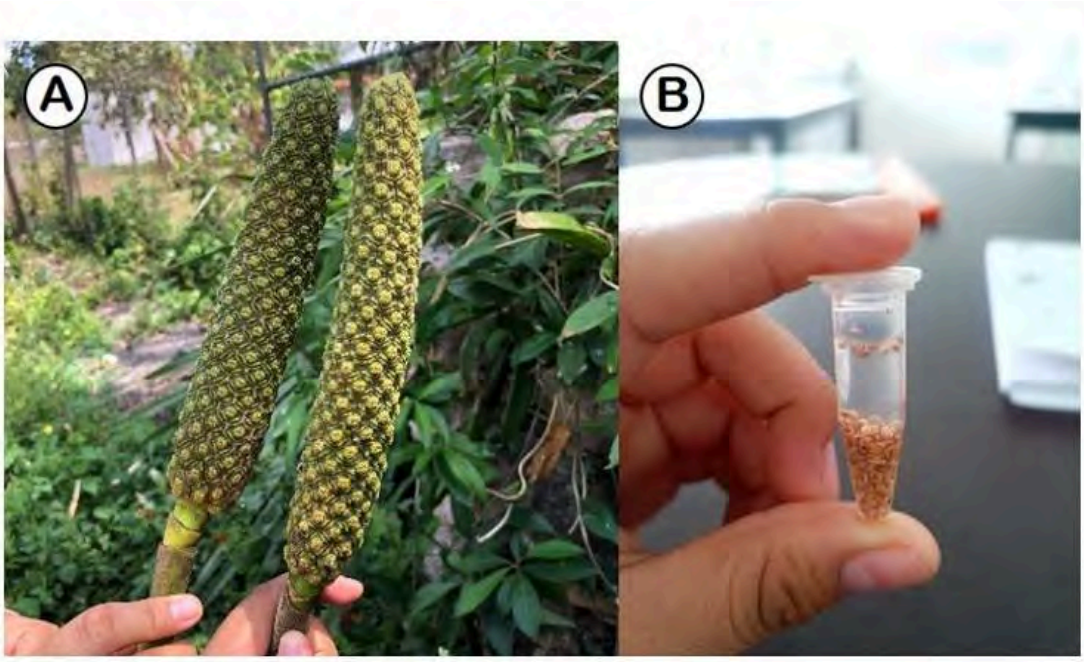
The toquilla palm (*Carludovica palmata*) is a phytogenetic resource in high demand in southeastern Mexico for its use in the production of handicrafts. It is grown only on small plots between Campeche and Yucatán (Muñóz-Sánchez *et al*., 2021), which generates a shortage of raw material. Its seeds germinate slowly in the field (Gómez *et al*., 2011), but *in vitro*, they achieve germination in 60 days or 21 days with gibberellic acid (GA3) (Zambrano-Arteaga, 2022). The use of 6-Benzylaminopurine (BAP) has promoted the formation of shoots, but the plants obtained are smaller (Hoyos-Sánchez *et al*., 2020), so better treatments are required to produce plants in less time and with better characteristics for adaptation in the field.

Colored LED lights improve *in vitro* culture by influencing photosynthesis and morphogenesis (Nhut *et al*., 2015), with red and blue LED lights being particularly effective for the growth of various species (Bello *et al*., 2016), so it is possible to expect a similar response in toquilla palm vitroplants. This study evaluated the effects of different treatments and phytoregulators on the germination and growth of *C. palmata* vitroplants to optimize their mass production.

The seeds were obtained from immature dark green and not very fleshy infructescences (stage E1, Figure 1A) (Zambrano-Arteaga *et al*., 2022), collected from plantations in Santa Cruz Ex-Hacienda, Calkiní, Campeche, Mexico, 90.239722 longitude and 20.398333 latitude. After washing the infructescences with running water and commercial antibacterial liquid soap, the berries were cut with a sterile scalpel to extract the seeds with tweezers, which were placed on absorbent paper for 3 days at 25 ±3 °C and stored in sterile microtubes until use (Figure 1B).

Figure 1. Plant material used in this study. A) specimens of mature infructescences in stage E1 collected from the locality of Santa Cruz Ex Hacienda, in Calkiní, Campeche, Mexico and B) recovered toquilla palm seeds.



For *in vitro* seeding, seeds were immersed in 70% alcohol for 5 min, rinsed with sterile deionized water, and then treated with 1% sodium hypochlorite for 1 min. After a final wash with sterile water, they remained in it until seeding. The culture medium was based on that reported by Hoyos-Sánchez *et al*. (2020); Zambrano-Arteaga *et al*. (2022) with some modifications. Each liter included 4.3 g MS medium, 2 mg L^{-1} BAP, 0.025 mg L^{-1} IAA, 30 g L^{-1} sucrose, 0.5 mg L^{-1} thiamine, 2 mg L^{-1} glycine, 0.5 mg L^{-1} pyridoxine, 0.04 mg L^{-1} GA3, 5 g L^{-1} activated charcoal, and 2.5 g L^{-1} of Phytagel™, with pH adjusted to 5.7. It was sterilized at 20 PSI for 20 minutes. Two hundred seeds were sown in

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20 bottles (10 seeds per bottle) and incubated at 25 ±3 °C with continuous light. Germination was evaluated every seven days for 30 days.

The tests to evaluate the effect of kinetin and the light treatments were carried out with the seedlings derived from the germination test. Three of them were placed in bottles with 25 ml of culture medium supplemented with 2 mg L^{-1} kinetin (KIN) instead of BAP, keeping the rest of the components the same. The bottles were incubated at 25 ±3 °C with different lighting arrangements for 120 days.

The effect of the different LED light arrangements was evaluated by using a shelf in which 1.2 m LED strips were arranged, with 90 LEDs m^{-1} of 18 W and 1400 lumens. The lighting intensity was measured with a Dawson meter (DSM155). The first level had red LED lights (65 lm m^{-2}), the second, blue LEDs (524 lm m^{-2}), the third, a combination of red and blue (393 lm m^{-2}), the fourth, white LED lights (654 lm m^{-2}) and the fifth, without light (0 lm m^{-2}) and with natural light insulation, using a thick black plastic for such effects. A shelf exposed to white fluorescent light (589 lm m^{-2}) was used as a control.

The plants were exposed to a 24 h photoperiod and their height, number of leaves, number of roots, and average length of roots and shoots were measured at 18, 50 and 120 days. Data were analyzed with a one-way Anova and Tukey's test (α= 0.05) using IBM SPSS Statistics 26 (IBM, 2019).

With the reported culture conditions, 85.5% germination was achieved after three weeks (Figure 2A). These results surpass those obtained by Zambrano-Arteaga *et al*. (2022), who reported emergence of cotyledonary leaves at 21 days, whereas this study obtained 34.5% germination in the first eight days.

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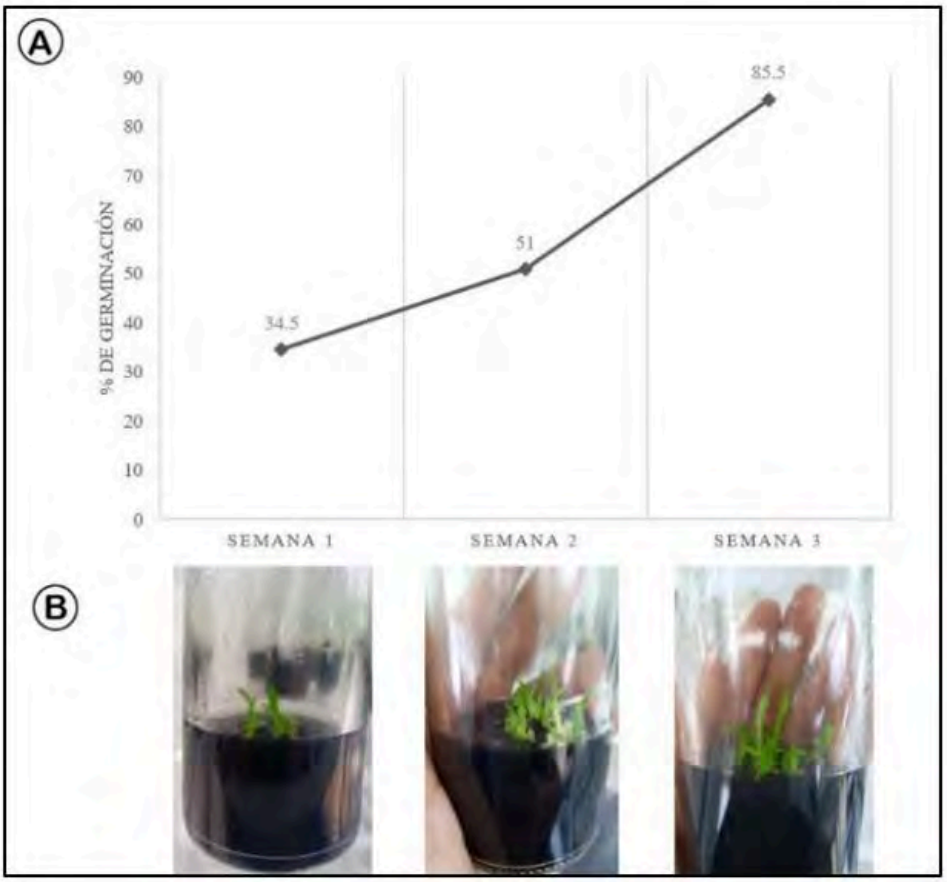
Figure 2. Germination and initial development of *C. palmata* in a modified medium (Zambrano-Arteaga *et al.*, 2022), with 2 mg L $^{-1}$ BAP, 0.04 mg L $^{-1}$ GA3 and 2 mg L $^{-1}$ AAI. A) percentage of germination over time and B) seedling growth after 21 days of experimentation.



Contrary to expectations, kinetin had no significant effect on the number of induced shoots in seedlings (data not shown). On the other hand, up to three months of incubation, differences between treatments began to be observed. White and blue/red (50/50%) LED lights showed positive effects, with the largest seedlings being those exposed to white LED light (Table 1, Figure 3D), similar to what was reported for the orchids *Oncidium tigrinum* and *Laelia autumnalis* (Murillo-Talavera *et al*., 2016) and the effects reported for *Vanilla planifolia* and *Anthurium huixtlense*, in which the combination of red and blue LED lights induced higher growth (Chen *et al*., 2013; Bello *et al*., 2016; Martínez-Estrada *et al*., 2016).

Table 1. Effect of LED lighting at different post-sowing times on the height of *C. palmata* seedlings

Lighting treatment	Post-sowing time		
	18 days	50 days	120 days
Red LED	0.9^{a}	2.94^{ab}	8.45^{b}
Blue LED	1.37^{a}	2.6^{a}	10.25^{b}
Blue/red LED	1.25^{a}	4.49^{b}	11.5^{bc}
White LED	1.12^{a}	2.96^{ab}	14.58^{c}
Darkness	1.76^{a}	1.42^{a}	4.4^{a}

Revista Mexicana de Ciencias Agrícolas

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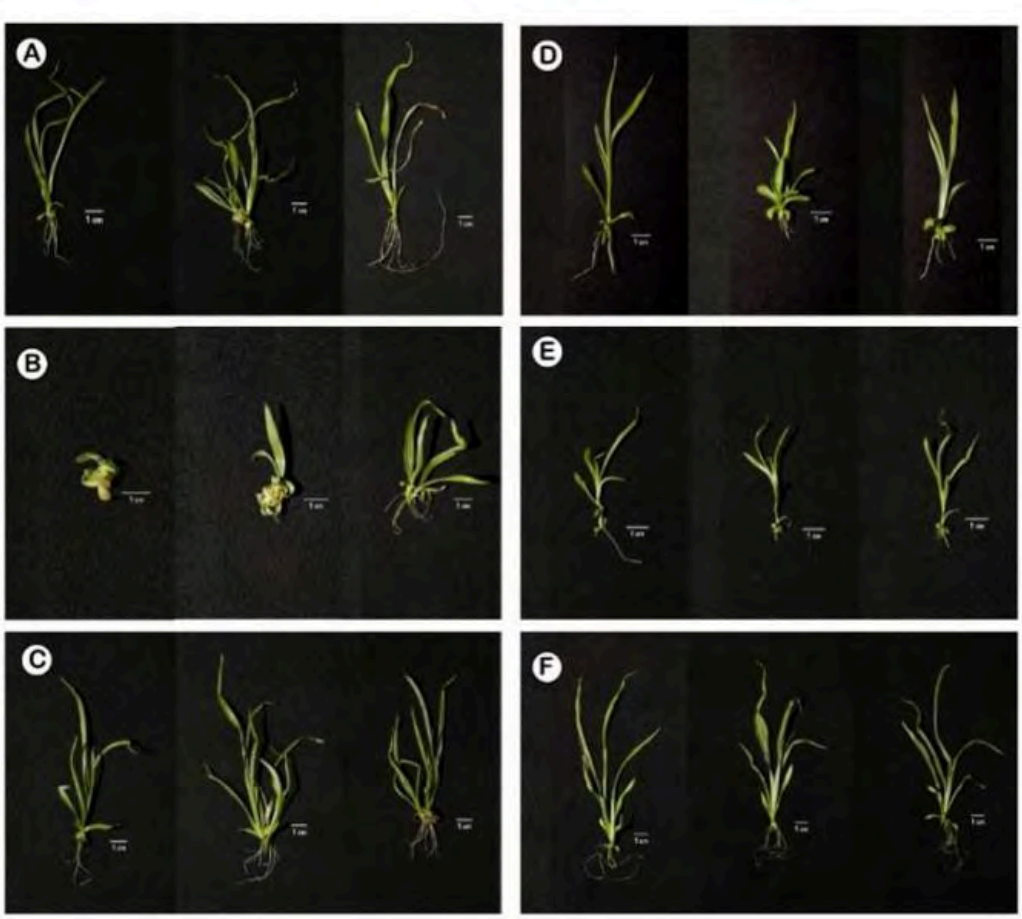
Lighting treatment	Post-sowing time		
	18 days	50 days	120 days
White fluorescent	1.68[a]	2.44[a]	10.94[b]

Different letters (a, b, c) in the same column indicate statistically significant differences (Tukey, $p \leq 0.05$).

Figure 3. Photographs of seedlings under different lighting arrangements after 90 days. Treatments. A) Blue LED; B) red LED; C) blue/red LED (50% each); D) white LED; E) darkness and F) white fluorescent light.



The red LED light produced longer roots, as reported in grape explants (Poudel *et al*., 2008) (Table 2). Similarly, a study by Wu and Lin (2012) showed that exposing *Protea cynaroides* seedlings to red LED light promotes a considerable increase in root density and length compared to conventional lighting conditions. Likewise, seedlings under blue/red LEDs developed more leaves and longer roots than those with white light, which favors their acclimatization (Figure 3C, Table 2).

Table 2. Effect of LED lighting on the growth of *C. palmata* vitroplants after 120 days of incubation.

Lighting treatment	Num. of shoots	Average root length (cm)	Num. of roots	Num. of leaves
Red LED	0.3[a]	8.6[b]	6.3[a]	6.3[ab]
Blue LED	0.6[a]	6.8[ab]	6[a]	7.6[ab]

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Lighting treatment	Num. of shoots	Average root length (cm)	Num. of roots	Num. of leaves
Blue/red LED	0.5[a]	7.3 [ab]	6.8[a]	10[b]
White LED	0.3[a]	5.3 [ab]	7.6[a]	8[ab]
Darkness	0[a]	1.75[a]	5.6[a]	4.6[a]
White fluorescent	0[a]	2.6[ab]	8[a]	6.4[ab]

Different letters (a, b, c) in the same column indicate statistically significant differences (Tukey, $p \leq 0.05$).

Conclusions

A more efficient *in vitro* germination protocol for *C. palmata* than those reported in previous studies was established. The use of LED light significantly increased seedling growth, with the combination of red and blue LEDs standing out, which promoted leaf formation, whereas red LEDs favored root elongation and white LEDs favored seedling elongation after three months of treatment.

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Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v16i3.3614

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Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Article/Issue Information
Date received: 01 January 2025
Date accepted: 01 March 2025
Publication date: 19 May 2025
Publication date: Apr-May 2025
Volume: 16
Issue: 3
Electronic Location Identifier: e3614
DOI: 10.29312/remexca.v16i3.3614

Categories

Subject: Investigation note

Keywords:

Keywords:

Carludovica palmata

in vitro culture

LED light

toquilla palm

Counts

Figures: 3

Tables: 2

Equations: 0

References: 12

Pages: 0